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Role of Cytosolic Phospholipase A_2 in Cell Injury

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Phospholipase A_2 (PLA_2) comprise a family of enzymes that hydrolyze the acyl bond at the *sn*-2 position of phospholipids to generate free fatty acids including arachidonic acid and lysophospholipids.

Distinct forms of PLA_2 are involved in digestion, inflammation, and intercelluar-and intracellular signaling pathways. The released arachidonic acid, which is enriched at the *sn*-2 position, serves as the precursor for eicosanoids such as prostaglandins and leukotrienes. During oxygenation of arachidonic acid to hydroxy endoperoxide, reactive oxygen radicals are generated. On the other hand, lysophospholipids increase membrane fluidity and can be cytotoxic with its detergent-like action. Thus, the biochemical features of the products of PLA_2 activity suggest that PLA_2 may be implicated in many destructive cellular processes.

To elucidate a possible role of cytosolic PLA_2 ($cPLA_2$) in cell injury, several experimental models were utilized: 1) glutamate-induced neuronal cell injury, 2) hydrogen peroxide (H_2O_2)-induced injury of LMPC17 cell line stably expressed with $cPLA_2$ in LLC-PK1 cell line, a kidney epithelial cell, 3) H_2O_2-induced injury of murine fibroblast (L-929) and its H_2O_2-R) cells, and 4) methyl mercury (CH_3HgCl)-induced injury of MDCK cell lines.

Firstly, glutamate stably enhances the activity of two cytosolic forms of PLA_2 in brain cortical cultures. We have directly measured PLA_2 enzymic activity in cell-free extracts from cortical neuronal cultures from rat brain and have found that the PLA_2 activity is up-regulated after cells are exposed to glutamate. Two Ca^{2+}-dependent forms of PLA_2 were identified in cytosolic extracts. Down-regulation of protein kinase C activity partially blocked glutamate's effects. One of the two forms was similar to the 100 kDa cytosolic PLA_2, and the other form of 13.5 kDa may be a novel brain PLA_2 whose activity was activated at lower Ca^{2+} concentration as a result of stable modification of the enzyme induced by glutamate. Thus, glutamate-induced stable enhancement of PLA_2 activity. by processes involving calcium and protein kinase C activation, is a potential molecular switch probably mediation changes in synaptic function and contributing to excitotoxicity.

To establish a causal relationship between the increase in PLA_2 activity and the cellular injury, we have used LMPC17 cell line which stably expresses $cPLA_2$ by 50-fold in LLC-PK1 kidney epithelial cell line. Exposure to H_2O_2 resulted in significantly greater lactate dehydrogenase (LDH) release in LNPC17 cell lines when compared with control cells. Furthermore, NDGA, an inhibitor of $cPLA_2$, significantly blocked LDH rease. This finding suggests that $cPLA_2$ may be an important mediator of oxidant damage to renal epithelial cells. On the other hand, as another experimental model of oxidative cellular injury we have characterized PLA_2 activity in murine fibroblast (L-929) and its H_2O_2-resitant mutant (H_2O_2-R)

cells and have examined a causative effect of $cPLA_2$ on their oxidant damages. After exposure of H_2O_2, the $cPLA_2$ activity of cell-free extracts from H_2O_2-R cells was lower by two-fold when compared with control cells. Treatment of [^{3}H] arachidonic acid ([^{3}H]AA)-prelabeled cells with 1 mM H_2O_2 for 30 *min* increased the release of [^{3}H]AA, and the release was higher by 3 to 4-fold in L-929 cells than H_2O_2-R cells. The releases of [^{3}H]AA and LDH were inhibited by antioxidant N-acetyl cysteine or mannitol, and by the treatment of a cytosolic PLA_2 activity and the reduced release of [^{3}H]AA in H_2O_2-R cells may cause its resistance to H_2O_2-induced cytotoxicity.

The relationship between methyl mercury (CH_3HgCl)-induced cytotoxicity and $cPLA_2$ activation was examined in Madin Darby Canine Kidney (MDCK) Cells. Methyl mercury is the most important form of mercury in terms of cytotoxicity and health effects from environmental exposures because of its high level of bioconcentration through a food chain in a member of organisms such as fish and the high absorption rate into organism. Despite the biological importance of methyl mercury the underlying mechanism by which it damages the cells is still poorly understood. The [^{3}H]AA release preceded LDH release and was inhibited by the pretreatment of a cytosolic PLA_2 inhibitor $AACOCF_3$, or N-acetyl cysteine. The methyl mercury-induced [^{3}H]AA release was independent of protein kinase C acitivity, but required Ca^{2+} ion in extracellular medium. This finding suggested that the release of [^{3}H]AA via $cPLA_2$ activation may be responsible for methyl mercury-induced injury of MDCK cells.

Finally, oxLDL has recently been shown to induce toxic effects in mouse peritoneal macrophages and human monocyte-macrophages *in vitro*. It is believed that the death of macrophage foam cells contributes to the lipid core in the advanced lesion of atherosclerosis OxLDL induces lipid peroxidation and depletion of cellular glutathione and ATP, which may be the consequence of oxidative stress. Recently, in cell death, oxLDL has been shown to interfere with signaling pathways involving calcium, CPP32, ceramide, manganese superoxide dismutase (MnSOD), and p53. However, at present, the precise signaling mechanism of oxLDL-induced cell death is not fully understood. On the other hand, it was reported that oxLDL increases arachidonic acid (AA) metabolites synthesis, just forcing inflammation during atherosclerosis. There are few studies on the relationship between oxLDL-induced AA release and phospholipase A_2 (PLA_2). Our results are as follows: 1) OxLDL, 7-ketocholesterol, and 25-hydroxycholesterol induced the releases of AA and LDH from murine macrophage RAW 264.7 cells in a dose- and time-dependent manner. 2) The oxLDL and oxidized cholesterols-induced releases of AA and LDH were inhibited by $AACOCF_3$, a specific inhibitor for $cPLA_2$, in a kinetically similar pattern, indicating the activation of $cPLA_2$. 3) It was suggested that the stable increase in the $cPLA_2$ activity is due to MAPK-mediated phosphorylation via activation of PKC as well as *de novo* synthesis of the enzyme. 4) OxLDL-induced foam macrophage cell death contributes to the activation of $cPLA_2$, which may be a target for the development of anti-atherosclerosis.

Taken together, $cPLA_2$ may cause various forms of cellular injury.